IN-SILICO DETERIORATION OF PLASTIC BY *DIETZIA MARIS* STRAIN CYTOCHROME P450 CYP153A16

ABSTRACT

Microbial degradation is a supportive method to treat the environmental pollution expanding due to excess production of plastic waste. Cytochrome P450 is highly effective enzyme for plastic degradation and many other pollutants due to vast range of substrate specificity. The present study was designed to predict the degradation affinity of cytochrome P450 for two types of plastic that were polycarbonate and phenol formaldehyde. It was also focused on identifying newly emerging harmful plastic for degradation and to determine their toxicity level. Maximum number of essential tools were applied to design this study of plastic degradation. The sequence of targeted strain was retrieved from NCBI and subjected to multiple sequence alignment and phylogenetic analysis. Protein model was generated and purified after the translation of gene into protein. Acidic and alkaline nature of enzyme was predicted through computational tool named as AcalPred. Active sites were detected and the protein was docked for polycarbonate and phenol formaldehyde individually. PyMol was operated for the visualization of receptor protein cytochrome P450 and ligands polycarbonate and phenol formaldehyde interactions. Effective energies were observed towards the degradation of polycarbonate and phenol formaldehyde. *Dietza maris* was observed more effective to degrade polycarbonate than phenol formaldehyde. Both types of plastic were found to be harmful as a result of toxicity analysis that was done through ECOSAR. The detailed interaction studies were predicted through a computational tool named as Discovery studio.

Gene cytochrome P450 was also cloned into the vector pUC-19 to predict the insertion of gene into some other organisms and to pursue it for downstream utilizations. The finding indicates that *in-silico* degradation method can be practiced to predict the degradation ability of any enzyme and *in-vitro* studies can be perused to detect the actual ability of enzyme for degradation.

CHAPTER ONE: INTRODUCTION

According to modern biotechnology, it is very recent approach to use living organisms to decontaminate the environment from harmful and non-degradable substances. Microbes play an important role in reducing the pollution of the environment via metabolic or enzymatic actions. The impact of artic melting is also important (Sakib, 2022). They do bioremediation to convert more toxic substances into lesser one or to remove it from the environment (Medina-Bellver *et al*., 2005). This method is more preferable over the chemical and burning methods. This is because of its cheapest price and less requirement of energy and equipment (Medina-Bellver *et al*., 2005). Microbes are natural source to decontaminate the environment. This method is applicable both on land and under water pollutant's treatments. It can also be used to treat the oil spills and petroleum-based products. Wastewater is polluting lakes, pounds and even sea have a lot of contaminated amount of waste which is hazardous for the acute life, dangerous for living things (Sakib, 2022).

Bioremediation is a cheapest and natural method to degrade many pollutants by the actions of various microbes (Shahnawaz *et al*., 2019). Microbes degrade more toxic substance into simpler substances to use them as their own source or to use it for some other purposes. The degradation takes place due to the metabolic or enzymatic activity of microbes. The process of bioremediation is affected by many factors such as nutrients, pH, availability of oxygen, composition of targeted material, chemical and physical properties of the pollutant (Margesin and Schinner, 2001). (Margesin and Schinner, 2001). Microbes works under specific pH and they need proper nutrients and oxygen to fulfill their task. Anaerobic microbes do not need oxygen for their metabolic reactions.

Microbes show ability of environmental bioremediation by enzymatic action or metabolic processes. They showed various enzymes that may degrade pollutants in the environment such as laccase in *Rhodococcus ruber C208*, esterase in *Proteus*, lipase in *Bacillus subtilis*, PETase in

Indeonella sakaiensisand and P450 in *Dietzia maris* etc. Some microbes showed the involvement of protein coding genes to degrade the material such as alkB gene in *Pseudomonas sp.* E4, alkB1 and alkB2 gene in *Pseudomonas aeruginosa E7,* etc. (Ru *et al*., 2020). There are many other genes and enzymes that are important for biodegradation. All strains have different mechanisms and reactions to do bioremediation.

P450 an enzyme with the greatest capability of catalyzing the reaction, developing biosensors, biosurfactant production etc. (Girhard *et al*., 2015). (Girhard *et al*., 2015). It can also use for the variety of oxidative reactions which include olefin epoxidation, hydrocarbons hydroxylation, desaturation of C-C bonds, heteroatoms oxygenation etc. (Vaz *et al*., 1998). There are various sources of P450. It is found in *Homo sapiens* in the form genes named as CYP2C8, CYP2C9 and CYP2C10. CYP102A1 is found in *Bacillus megaterium*. *Bacillus subtilis* 168 contains P450 genes named as cypB and cypD.

Cytochrome P450 Cyp153A16 is found in *Dietzia maris* which have capability of degrading various polymers. *Dietzia maris* can be found from various sources such as soil, degrading oil field, skin of carp and artiular fluid of human's knee. It is linked to actinomycetes gram-positive family with high GC contents. *Dietzia maris* contains a proteins coding gene cytochrome P450 Cyp153A16 with the size of 1389bps. P450 cytochrome contains variety of thiolate legated heme proteins which reduce equivalents to catalyze the oxidative reactions. The equivalents are derived from molecular oxygen, NADH or NADPH (Vaz *et al*., 1998). The melting point of P450 protein is 40-45^{o}C and its pH for catalytic activity ranges from 6-9 (Anzenbacher *et al*., 1982).

Today, a large quantity of different types of pollutants are being produced due to various human activities such as waste plastic (Chae and An, 2018), microplastic like PEG (Xia *et al*., 2020), use of private vehicles toxifying the air (Zhang and Batterman, 2013), emission of organic gases (Mølhave, 1982), drug pollution (Larsson, 2014), pesticides (Thompson, 1973), health risks due to aromatic hydrocarbon pollution (Wang *et al*., 2011), petroleum-based products like petroleum

hydrocarbons (Pinedo *et al*., 2013), oil pollution (Sakib, 2021), and other industrial waste. These are newly emerging pollutants which are harming the living and non-living environment. There is a need to detoxify pollutants by new methods which can protect the environment from any damage. Industries are played important role in developing our life styles but the piling of various pollutants is causing damage to the environment. They are not only damaging the environment but also the biological life in different terms. They are accumulating in the soil to damage the plants. Carcinogenic effect of pollutant can damage the human immune, respiratory and nervous system. Thus, these are need to be treated or removed from the environment with proper methods. Plastic which is a need of time is great health hazard. People use to waste plastic after use and don't try to recycle it. It is undegradable material as it is made up of polymers. Mostly plastics are used to convert into microplastics that are dangerous both in case of further use like in cosmetics and discarding. Some waste plastic become a part of soil to damage soil and plants and some are disposed into the oceans to disturb the marine life (Chae and An, 2018). It causes damage to marine life by inducing variety of diseases thorough toxins, bacteria, viruses etc. It can also harden the bottom surface and make it resistant to the exchange of oxygen gas (Gerber, 2015). It lessens the fertility of soil and it can change the chemical composition of soil. It may induce toxic substances into the soil which definitely cause damage to animal's life if are engulfed in the form of eatables. As plastic is undegradable, if it is burned in an open air, it directly releases various toxic substances to pollute the air.

Polycarbonate and phenol formaldehyde are the most common plastic using today for various lenses, medical apparatus, greenhouses, lighting fixtures, antennas etc. Polycarbonate is derived from the monomers of Bisphenol A and phenol formaldehyde is derived from Bakelite polymer. They have variety in terms of low and high density. They are made up of polymers. They are toxic because they are non-degradable. Chemical degradation and combustion pollute the environment. Such methods of degradation are again harmful or not helpful to reduce the problem.

There is a need to degrade polycarbonate and phenol formaldehyde after use so that we can protect the aquatic life which stops the entry of them into the food chain to ultimately protect the human's health. Plastic waste from textile industry, packaging industry, house hold wares, automotive industries, toys, bottles, plastic pipes etc., all are non-degradable. Plastic is mostly hydrophobic in nature so does not absorb by water. On combustion, plastic releases harmful gases which includes furans, dioxins, bisphenol, mercury and many other harmful gases. Dioxin interferes in human health by causing respiratory problems, causing damage to immune system, cancer (Kogevinas, 2001). Furans are more common in causing skin problems and all other gases release on the burning of plastic are dangerous for both environment and humans. Plastic can directly damage the aquatic life and humans. If it is not treated, it would cause a great damage to our Earth. Rural Uganda is facing problems with clean water (Sakib, 2021).

Cytochrome P450 with the combination of genetically modified plant has been used for the degradation of low molecular weight organic compounds (James *et al*., 2008). Benzoic acid has also been degraded by using cytochrome P450 (Ning *et al*., 2010). It also takes part in the degradation of oil by producing de-rhamnolipid which serves as biosurfactant (Wang *et al*., 2014a). It can also degrade petroleum-hydrocarbons by using it as carbon source (Wang *et al*., 2011). It can degrade alkenes by epoxidation and hydroxylation method. In the same way it has been proving good for decontaminating the environment.

Cytochrome P450 has vast range of substrate specificity. It's a need of time to predict more pollutants that can be degraded by using this enzyme. We can use *in-silico* approach to fulfill this purpose. It is cost effective and time saving method before *in-vitro* work. This study focuses on the prediction of pollutants that are degradable by using P450. The toxic effects of pollutants must be known. P450 is an enzyme which has many eukaryotic and prokaryotic sources to degrade the pollutants such as oils and polymers. While discussing about the public health perspective, analytical method for marginalized group by Sakib (2022) was followed.

Research Objectives

Followings aims and objectives were considered in this study.

- To determine the degradation of Polycarbonate and Phenol formaldehyde using cytochrome P450.
- To study the secondary and tertiary structures of the protein Cytochrome P4550.
- To screen new effective strain for plastic degradation to decrease plastic pollution.
- To detect the toxicity of pollutants, Polycarbonate and Phenol formaldehyde.

CHAPTER TWO: REVIEW OF LITERATURE

2.1 Polymers

Polymers are the long chain molecules which are can be both natural and synthetic and are made up of the joining of monomers in a long chain (Mutlu and Lutz, 2014). Nowadays, natural polymers are rapidly replaced by synthetic polymers and plastic have grown to an important part of our lives (Shah *et al*., 2008). Organic and inorganic sub substances like oxygen, carbon, chloride, hydrogen and silicon are used for the production of plastic and these materials are derived from oil, natural gas and coal (Seymour, 1989). Microbes are unable to degrade plastic due to very short term presence in nature and lack of plastic degrading enzymes (Abou-Zeid *et al*., 2001).

2.2 Structure of Plastic

Plastic consists of polymers which are high molecular wight molecules made by the joining of low molecular wight monomers in a long chain. Polymers contain carbon, hydrogen, oxygen, nitrogen and silicon. The long chain of polymer is a part of backbone of the molecule in which many repeating units are arranged. This long chain polymer can be changed to change the properties of certain plastic (Charlesby, 2016). Structure of plastic can be changed by various methods. One of the reported methods is the material system in which the properties of plastic structural components are changed by treating with a medium. This medium produces homogenous mixture with the components of plastic with the addition of partial diffusion of plastic components (Ederer *et al*., 2013).

2.3 Synthetic and Natural Polymers

Synthetic polymers are very common in the society in the form of plastic and are very hard for biodegradation and causing environmental pollution in the form of plastic waste (Shimao, 2001). PE, PS, PP, phenolics etc. are the examples of synthetic polymers. Natural polymers have priority over synthetic polymers because they are derived from biological origin and are of low cost.

Also, they are easily biodegradable and are suitable for the environment (Malinconico *et al*., 2014). Natural polymers include cellulose, wool, starches and rubber. Examples of natural polymer is polycarbonate.

2.4 Types of Plastic

There are various types of plastic that is transferred to disposing sites after use and the clean sites are replaced by mountains of plastic waste (Schwarz *et al*., 2019). Plastic is categorized into different types on the bases of its origin of production that includes natural and synthetic origins (Alshehrei, 2017). It can also be divided on the basis of its degradation methods such as thermal degradation and photodegradation (Fotopoulou and Karapanagioti, 2017). Polyethylene, polypropylene and polystyrene are the first three most commonly using plastic polymers in the society (Weinstein *et al*., 2016). Some other categories of plastic using in the society are polycarbonate, phenol formaldehyde, polyamide and polyvinyl chloride.

2.4.1 Polyethylene

Polyethylene is a synthetic plastic produced from petroleum-based origin and is very common plastic in bottles, bags, packaging, films, containers etc. It can be produced from natural gas. It is a plastic with high ductility, toughness and little friction. It has low melting point and available in low- and high-density polyethylene plastics. Polyethylene is also a favorable insulator. It is produced by the polymerization of ethylene in the presence of catalyst. Waste management is very difficult due to high usage of polyethylene plastic and un-degradability (Lin and Argon, 1994).

2.4.2 Polycarbonate

Polycarbonate is a synthetic and thermoplastic polymer with carbonate group found in its chemical structure. It is mostly used in engineering due to its toughness and strength. It is also very common in lenses due scratch- resistant property. It is used in constructive materials like green houses, CDs and DVDs, automotive, lab goggles and mobile phones. also used in. It

contains bisphenol A which is a man-mad organic material. It can be hydrolyzed into Bis-phenol A by providing high temperature (Kambour, 1964).

2.4.3 Polyamide

Polyamide is type of plastic which can be of biological origin like protein, wool and silk or artificial origin such as aramids, nylon and aspartate. Polymer composites that are alternates of plastic can be produced by using polyamides (Bhattacharyya *et al*., 2009). Almost all types of plastic have mineral fillers polymer composites are free from mineral fillers It is produced by the combination of two compounds, first is an amino group and second is terminal carbonyl group. It is classified into aliphatic, aromatic and polyphthalate (Ki and Park, 2001).

2.4.4 Polyvinyl Chloride

Polyvinyl chloride is a synthetic polymer made up of monomers of vinyl chloride via polymerization. It mostly contains plasticizers which make it more soft and more flexible. It has high molecular weight but it can be reduced by proving high temperature. It is used in pipes, electric cables, clothing, construction, flooring etc. It can be converted into low molecular weight microplastic by the action of microbes (Galgani *et al*., 2018). Its structure can also be modified by treating with Poly (N, N-dimethylacrylamide) brushes that are hydrophilic polymers (Zou *et al*., 2009).

2.4.5 Polystyrene

Polystyrene is a polymer derived from biological origin like cutin from cuticles of plants or synthetic like polybutyrate. All-natural polystyrenes are biodegradable while some of the synthetic polymers can be degraded by microbes. It can be aromatic or aliphatic in nature. It is used in clothing, bad sheets, furniture and mouse mats. Plastic bottles, guitars some parts of vehicles also contain polystyrene. It is non-renewable but some of its types can be degraded by microbial action.

2.4.6 Phenolics

Phenolics is a second name of phenol formaldehyde which is produced by treating phenol with formaldehyde. It is produced by polymerization with the addition of acid or base catalyst. It can be transformed by various reactions such as addition of epoxy group on hydroxyl group, introduction of cyanate esters, addition of benzoxazine and the reaction with compatibilization agents etc.. (Pilato, 2010). It is mostly used in laminations, loudspeaker drivers, billiard balls etc. It is degradable by microbes like *Phanerochaete* fungus. There is also an updated version of phenolics with higher modulus and more heat strength (Pilato, 2010).

2.5 Hazardous Effects of Plastic Waste

Plastic pollution is expanding everywhere and causing serious issues in the society which includes human health problems, contamination in food chain, water pollution, land pollution, air pollution, death of various animals, expensive treatments etc. (Verma *et al*., 2016). All these factors are causing environmental pollution and inducing chemicals into the plants and animals. These chemicals are totally carcinogenic, neurotoxic and hormone-disturbing chemicals which are disturbing their normal metabolism and causing lethal effects.

2.6 Decomposition Methods of Plastic Waste

There are some harmful methods of plastic decomposition which include landfilling, incineration and marine disposal (Alfarisi and Sutopo, 2019). These are the most common methods but are the cause of many hazards such as land pollution, soil fertility damage, induction of harmful chemicals into the plants, air pollution, marine pollution and many other health hazards. It is also contaminating the food chain of human entering by entering into the plants and animals (Toussaint *et al*., 2019).

2.6.1 Landfilling

Almost 10% of plastic waste is generated from households out of which the heavy quantity of plastic waste is thrown on landfill sites (Verma *et al*., 2016). One of the harmful chemicals which

is released due to the leaching of plastic waste is bisphenol A which is a cause behind many health issues (Yamamoto *et al*., 2001). The major disadvantage of landfill is the lack of reusable plastic recycling and the whole plastic waste is discarded on landfill without considering its harmful effects on the environment and animals (Siddiqui and Pandey, 2013). Landfill waste can be utilized for the generation of energy which can drop down the level of increasing global warming (Zuberi and Ali, 2015).

2.6.2 Incineration

Incineration involves the burning of plastic waste at landfill sites and it produces harmful byproducts in which carbon dioxide, carbonic acid and water are included (Verma *et al*., 2016). Incineration releases toxic compounds which are hazardous for environment and human health such as acetone, benzol, phosgene, acetaldehyde, xylene and toluene etc. They can cause various serious issues such as immunity disturbance, damage nervous system, skin allergies, respiratory problems and can affect liver and bone marrow (Ágnes and Rajmund, 2016).

2.6.3 Marine Disposal

Around 60-80% of the waste plastic is disposed into the ocean (Verma *et al*., 2016). Marine environment and animals are largely affected by plastic pollution. It has been reported that some marine animals such as fish, turtles and seabird, have exposed plastic waste during research (LI *et al*., 2016). This waste plastic can block the intestinal tract, damage the secretion of various essential enzymes and cause abnormality in reproduction system (Azzarello and Van Vleet, 1987). Another bad effect of marine plastic pollution is entanglement. 55% cases of marine animals' entanglement have been recoded (Barboza *et al*., 2019). The death rate of marine animals such as fish, turtles, seabirds and mammals is increasing due to this entanglement (Gilman *et al*., 2010).

2.7 Degradation of Plastic

Environmental sources like moisture, light, heat, biological action and chemical changes can diversify the physical or chemical nature of polymers and these changes causes the deterioration

of plastic due to bond cleavage (Pathak, 2017). Many synthetic plastics are degraded by photolytic reaction, thermo-oxidative reaction, photo-oxidative reaction and by absorbing UV radiations from solar energy (Singh and Sharma, 2008). The degradability of natural plastic is dependent upon the components found in polymers and it can be degraded by the microbial or enzymatic action (Adhikari *et al*., 2016). Oxo-biodegradation, biological degradation and enzymatic degradation involve microorganisms which degrade plastic polymers by applying different methods.

2.8 Types of Degradation

Some major divisions of plastic degradation include photodegradation, thermal degradation, oxobiodegradation, biological degradation and enzymatic degradation. Environment mostly offers photodegradation by providing ultra violet rays to degrade the higher molecular weight plastic waste into the low molecular weight type (Yousif and Haddad, 2013). Thermal degradation of plastic requires high amount of thermal energy to break the polymer linkages (Yu *et al*., 2016)

2.8.1 Photodegradation

Photo-degradation depends upon the ability of polymers to take in the affective radiations which are responsible for polymer degradation. Affective radiations include Ultraviolet-A radiation of wavelength ~315–400 nm, Ultraviolet-B radiation of wavelength ~295– 315 nm and infrared radiation of wavelength 760– 2500 nm (Pospíšil and Nešpůrek, 1997). Photo-degradation is carried out through the activation of electrons due to absorption of high-energy radiation (Shah *et al*., 2008).

2.8.2 Thermal Degradation

Thermal degradation causes deterioration of plastic by providing high heat which changes the chemical and physical properties of polymers by breaking down of long chain of back-bone molecule (Shah *et al*., 2008). During thermal degradation, depolymerization initiates from weak

bonds of the polymer (Singh and Sharma, 2008). Thermal degradation includes reducing the molecular weight, changes in color, decrease ductility of material and crashing (Olayan *et al*., 1996).

2.8.3 Oxo-biodegradation

Oxo-biodegradation includes two processes which are oxidation and thermal photodegradation. Oxidation degrade plastic by providing heat and photodegradation cleaves the end product by providing UV light. Both processes lower the molecular weight of plastic for degradation (Shah *et al*., 2008). Collectively, oxidation reaction and microbial attack on polymer is also known as oxo-biodegradation. Polyethylene has been degraded via oxo-biodegradation and it has been reported that the oxidation products are ecofriendly because they are biodegradable (Wiles and Scott, 2006).

2.8.4 Biological Degradation

Micro-organisms are used for deterioration in biological degradation and it includes aerobic and anaerobic degradation that are with and without oxygen respectively. Aerobic biodegradation produces water and carbon dioxide while anaerobic biodegradation produces water, carbon dioxide and methane (Chaisu, 2016). Microbes converts high molecular polymers to low molecular weight monomers and produce wastes which are further consumable by some other microbes (Shah *et al*., 2008).

2.8.5 Enzymatic Degradation

Enzymatic degradation includes two methods, first is attachment and second is hydrolysis. It involves intracellular or extracellular degradation. Endogenous carbon is consumed during intracellular degradation and bacterial aggregation is crucial for this type of degradation while endogenous carbon is utilized for intracellular degradation and bacterial accumulation is not important for it (Tokiwa and Calabia, 2004). Enzymes that are released by microorganism for polymer degradation are of two types and these are an endoenzyme and an exoenzyme (Raziyafathima *et al*., 2016).

2.9 Plastic Waste Management at Individual Level

Plastic pollution is increasing due to different human actions such as extraordinary use of plastic products, non-degradability, disposing of plastic waste anywhere any other human activities are also included. Plastic waste management at individual level can treat the plastic pollution and give good results. One should have to control the plastic use for common purposes and should discover some alternative methods like cloth bag. Another method is to reuse of plastic like waste plastic bottles for decoration pieces. We have to convince careless persons to control the plastic pollution or have to do campaign in the societies to spread awareness about the harmful effects of plastic pollution.

2.10 Enzyme Mediated Deterioration

Different pollutants are causing damage to environmental due to human activities. New methods of plastic degradation like landfilling, incineration and marine disposal are the major cause of plastic pollution. Plastic wastes destroy lands, add harmful chemicals in the soil, release harmful gases on incineration and water pollution. In this way, the plastic pollution is entered into the human beings, animals and plants and disturb their normal life. Thermal cracking is an effective method to degrade plastic but it requires more energy and not beneficial for economy. An alternative method is to use micro-organisms for the management of pollution. Microbial degradation is an environment friendly method to treat the pollutants. It is also a cost-effective method of plastic degradation. Microorganisms also present some effective enzymes for the degradation of pollutants. Enzymatic degradation is an effective approach for waste treatment.

2.10.1 Dietzia maris strain P450

Cytochrome P450 is found in *Dietzia maris* As-13-3 obtained from hydrothermal area of deep sea. It has been used in biosurfactant for the degradation of oil (Wang *et al*., 2014b). Cytochrome P450 has shown effective results for the oil degradation. It has also been used for degradation of petroleum-based hydrocarbons ranged from C6t to C4 (Wang *et al*., 2011). A number of

pollutants have been deteriorated by *Dietzia maris* Cytochrome P450 and many are yet to be discovered.

2.10.2 Molecular Docking

Molecular docking is a computational method to determine the natural sites, orientation and recognition of ligands such as pollutants, binding with the residues of targeted polymers (Zoete *et al*., 2009). It is a very common and effective method for the identification degrading ability of any enzyme towards different pollutants (Morris and Lim-Wilby, 2008). It is a promising *insilico* way for carrying out degradation in which degradation is based upon the binding affinity of pollutant and enzyme.

2.10.3 Expression in Microorganism

Microorganisms have been used to determine the expression of any enzyme or protein isolated microorganism or any ither living organism such as plants or animals (Kuiper *et al*., 2004). For expression of prokaryotic gene in microorganisms, it is fundamental to know the coding regions both in gene and vector for correct cutting and insertion. Suitable vector selection is very important and it is chosen on the bases of gene size which is to be inserted (Lai *et al*., 2006). Expression analysis in microorganism is a crucial step in case the degradative enzyme has to be produced industrially.

CHAPTER THREE: MATERIALS AND METHODS

This study was carried out utilizing *in-silico* method and the work was done during the time period of plastic pollution to free the enticement from plastic waste. On the basis of an effective degradation ability, protein coding gene Cytochrome P450 was preferred for waste plastic degradation. It had been used for oil degradation before and in this work same strain was utilized for plastic degradation. Detailed studies were done and all essential tools and software were utilized.

3.1 Sequence Retrieval and Basic Local Alignment

Polycarbonate and phenol formaldehyde degrading bacteria *Dietzia maris* strain cytochrome P450 cyp153A16 (accession number: KP202088) sequence size of 1839bp, was retrieved from NCBI, the online data base National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) (Akhter *et al*., 2017). Sequence of gene was downloaded in FASTA format. Gene sequence was used to find out sequence similarity of homologous sequences and family domains to predict evolutionary relationship among targeted strains (Selvitopi *et al*., 2020). FASTA sequence was subjected to local sequence alignment using BLASTn. Top seven strains with percentage identity >80% and query coverage >90% were chosen and downloaded in FASTA file.

3.2 Multiple Sequence Alignment and Phylogenetic Analysis

Multiple sequence alignment plays an essential part to enlighten the correlations among sequences and also highlight the conserved regions among species (Gotoh, 1999). FASTA file of aligned sequences were subjected to multiple sequence alignment using CLUSTALW to analyze the ancestral history of *Dietzia maris* strain P450 (https://www.genome.jp/toolsbin/clustalw) (Thompson *et al*., 2003). Phylogenetic tree was constructed to interpret and to glean the evolutionary relationship of the targeted strain.

Sequence homology and phylogenetic analysis was done through MEGAX which is a platform to study molecular genetics and to analyze the ancestral history of certain strain (https://www.megasoftware.net/) (Kumar *et al*., 2018). FASTA file of seven homologous strains including targeted strain was downloaded. MEGAX was used to create new alignment file for DNA phylogenetic analysis. The whole sequence was selected and aligned by CLUSTALW. All the gaps between the sequences were deleted for better results because pairwise deletion can cause MEGAX to not calculate the distances between pairs correctly. Saved file was open

through Data and the correct alignments were selected. The sequence was subjected to the final step of Construction neighbor-joining tree in the Phylogeny for the final prediction of results.

3.3 Translation of Gene into Protein and Detection of Protein's Nature

ExPaSy is a portal which is linked to many tools and databases such as translate, uniport, Swiss tree, BLASTn etc. .(https://www.expasy.org/) (Artimo *et al*., 2012). It shows results in 6 operating frames in which our desired protein is present. FASTA sequence of gene cyp153A16 was translated into protein using ExPaSy-Translator. Longest sequence was selected starting from methionine and ending at stop codon. It is important to analyze the nature of protein as the activity of enzyme is largely affected by pH value (Lin *et al*., 2013). The type of enzyme either it is alkaline or acidic, was predicted through AcalPred (http://lin-group.cn/server/AcalPred) which is a sequence-based tool for discriminating between alkaline and acidic enzyme (Lin *et al*., 2013). Nature of protein, either it was acidic or alkaline, was detected by giving FASTA sequence of protein to AcalPred. It is played an important role as the activity of an enzyme is affected by the variation in pH.

3.4 Functional Domain Prediction and Identification of Motifs

Functional prediction is an important step to predict the major functional aspects of protein. Uniprot (https://www.uniprot.org/) was selected for the function prediction of *Dietzia maris* P450. Uniprot is a universal source to find out any information about protein including homology, functions, taxonomy, homologues, family domains and interactions (Consortium, 2014). Motifs identification is an additional but the beneficial step to predict the functional importance of protein. Motifs are the signature of protein families and they help to predict the protein functions. They are involved in the catalytic activity of enzymes. Motifs were predicted taking advantage of InterProscan (https://www.ebi.ac.uk/interpro/search/sequence/). It's a platform raised from various methods such as Prodom, PRINTS, Pfam etc. These all are methods used for the perception of protein signature (Akhter *et al*., 2017).

3.5 Secondary Structure Prediction

Secondary structure prediction is an essential step to predict the main structural features of protein that are helix, strands, loops and coils. Secondary structure is also essential for the prediction of binding affinity of protein (Maris *et al*., 2005). PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) was applied for the prediction of secondary structure as it is most preferred one for meticulous prediction (McGuffin *et al*., 2000). FASTA sequence of protein was retrieved from NCBI using accession number AJD81704 and submitted to PSIPRED.

3.6 Model Generation and Refinement

Generation of tertiary structure has vast importance during Insilco work. I-Tasser (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) was adopted to generate the tertiary structure of protein. It is a protocol and highly recommended for protein tertiary structure prediction (Zhang, 2008). The sequence of translated protein was submitted to I-Tasser (Iterative Threading ASSEmbly Refinement) to predict the structures as well as the functions of protein. Refinement was done by applying GalaxyRefine (http://galaxy.seoklab.org/cgibin/submit.cgi?type=REFINE). Protein refinement develops the relative models of protein that are structurally distant homologues (Park *et al*., 2018).

3.7 Protein's Properties Statistics and Hydropathy Plot

Some nucleotides play important part in the biological action of protein, protein's properties statistics were computed by software from EMBOSS named as Pepstats (https://www.bioinformatics.nl/cgi-bin/emboss/pepstats) (Wang *et al*., 2017). It gives results of all types of residues found in molecule with their total number and mol%. Hydropathy plot was drawn through Pepwindow(https://www.ebi.ac.uk/Tools/seqstats/emboss_pepwindow/) (Teymournejad *et al*., 2012). It predicts the hydrophobicity along the length of the sequence. FASTA sequence was given to both tools for the prediction analysis.

3.8 Model Validation and Ligands Selection

Ramachandran plot was generated by making use of PROCHECK (https://servicesn.mbi.ucla.edu/PROCHECK/) to analyze the stereochemical properties of protein(Laskowski *et al*., 1993). It gives results in the form of plots and comprehensive residue listening. RC plot gives the knowledge about torsion angles and it is also a model validation source which predicts the information about the residues of protein (Sobolev *et al*., 2020). Ligands that were polycarbonate and phenol formaldehyde were retrieved from PubChem and downloaded in SDF file (Karthika *et al*., 2020).

3.9 Toxicity Prediction of Ligands

Ecological Structure Activity Relationships (ECOSAR) is a software used for the toxicity prediction of harmful molecules towards the aquatic animals (Frank *et al*., 2009). It gives Log Kow value which predicts the ability of compound to attach on the soil surface or on living organism. It is directly proportional to molecular weight and inversely proportional to solubility in water. The PDB file of polycarbonate and phenol formaldehyde were converted to SMILES by Babel GUI which is a converter use for the conversion of SDF file to MOL or SMILES format (O'Boyle *et al*., 2011). The SMILES format of polycarbonate and phenol formaldehyde were opened in ECOSAR for aquatic toxicity prediction.

3.10 Molecular Docking

Cytochrome P450 Cyp153A16 was docked with polycarbonate and phenol formaldehyde individually. Molecular Docking was done via Auto dock Vina (Trott and Olson, 2010). First the P450 Cyp153A16(receptor) and polymers (ligands) files were converted to PDB format and saved using Discovery studio. PDB file of receptor was open in Auto dock vina and file was saved as macromolecule after adding polar hydrogen. The grid was set and saved as a text file PDB file of protein was saved as pdbqt file.

Polycarbonate and phenol formaldehyde were added one by one with the protein molecule and the number of torsions were set as 4 and 1 respectively. The files were saved as pdbqt file separately. Pdbqt files of ligand and receptor was cut paste in vina folder of C-drive. Configuration file was created according to the grid out-puts that were 70 size of x, y and z axis and their centers were 71.564, 71.366, 71.369 respectively. Exhaustiveness was set as 8. Command prompt was run for docking. Docking gives 9 different interactions of receptor and ligands in the form of energies from which we can detect the results.

3.11Visualization and Interaction Studies

The results of interaction were visualized using PyMol which is a molecular visualizer (Seeliger and de Groot, 2010). PDQBT file of out-ligands were open one by one with protein to foresee the interactions between ligands and protein. Non-bonded interactions are important in molecular identification and biological reactions (Gao and Huang, 2011). Discovery studio was adopted to check the targeted residues of P450 interacting with polycarbonate and phenol formaldehyde with highest energies observed as docking results.

3.12 Cloning and Expression Vector

Cloning was done through software named as Snap-gene (https://www.snapgene.com/snapgeneviewer/). Snap-gene is a computational tool which is very common for the gene cloning and the determination of expression of gene in a vector (Matsye *et al*., 2012). Plasmid PUC19 was imported from online snap-gene sequence and modified by adding two restriction sites Bpu10I and EagI. Restriction sites were added just after origin of replication to check the maximum cloning results. Targeted gene was inserted in plasmid and the final clone was seen though history.

CHAPTER FOUR: RESULTS

4.1 Sequence Retrieval and Basic Local Sequence Alignment

FASTA file of gene was used to analyze the homology with other sequences using BLASTn (Zhao and Chu, 2014). The seven sequences were selected that showed the percentage identity >80% and query coverage >90%. The homologous strains with their accession numbers are given in the Table 4.1. The most similar one observed was HDW12B chromosome from *Norcardioides.sp.* It showed percentage identity around 88.93%. This similarity showed that the strain may have similar structures and functions with some variation to *Dietzia maris* P450.

Table 4.1: *Homologues of Deitzia maris strain cytochromeP450 Cyp153A16*

Ser.No	Organism	Gene/strain	Accession number	Query coverage	Per.Ident
1.	*Norcardioides.sp*	HDW12B chromosome	CP049867.1	99%	88.93%
2.	*Gordonia sp.*	X0973 chromosome	CP054691.1	98%	82.34%
3.	*Gordonia iterans*	Co17 chromosome	CP027433.1	98%	82.34%
4.	*Gordonia sp.*	YC-JH1 chromosome	CP025435.1	98%	82.34%
5.	*Mycolicibacterium arabiense*	JCM 18538 DNA	AP022593.1	95%	81.80%
6.	*Rhodococcus erythropolis*	PR4 plasmid pREL1 DNA	AP008931.1	95%	81.75%
7.	*Nocardioides marinisabuli*	DSM 18965 chromosome	CP059163.1	93%	81.83%

4.2 Multiple Sequence Alignment and Phylogenetic Analysis

Multiple sequence alignment was checked through CLUSTALW (Hung and Weng, 2016). There were 7 groups start of multiple sequence alignment. These groups were the same aligned sequences that were selected in BLASTn. The alignment score between these groups were given in the form of ratio. The alignment score between the sequences of group 1 and group 2 was 23750. Group 2: Group 2 showed the alignment score of 26087 and group3: group 3 showed the same results as with the previous case. Group 4: Group 4 showed the score 23056 as the sequence alignment score. The sequence alignment score for Group5: group 6 was 21790. Group6: Group7 showed alignment score 20810 and the total alignment score was 180817. All these strains showed alignment score more than 200. Short lines show that the query coverage is not complete. The results of these top 7 strains in the form of graphical representation is shown in the Figure 4.1.

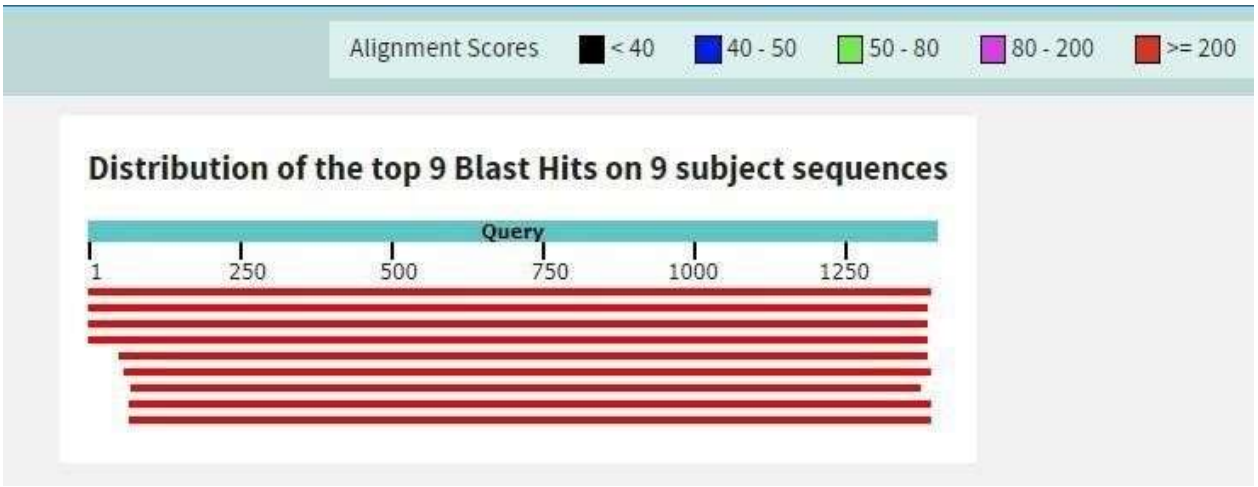


Figure 4.1: Graphical representation of aligned strains with cytochrome P450 cyp153A16

Then seven homologues were utilized to construct phylogenetic tree. Tree was constructed using a computational tool MEGAX and it showed maximum alignment with *Norcardioides sp.* Strain HDW12B chromosome. It showed 99% query coverage with percentage identity around 88.93%. Names of taxon were used to written in blue and that of branch information in red. Phylogenetic tree is shown in the Figure 4.2.

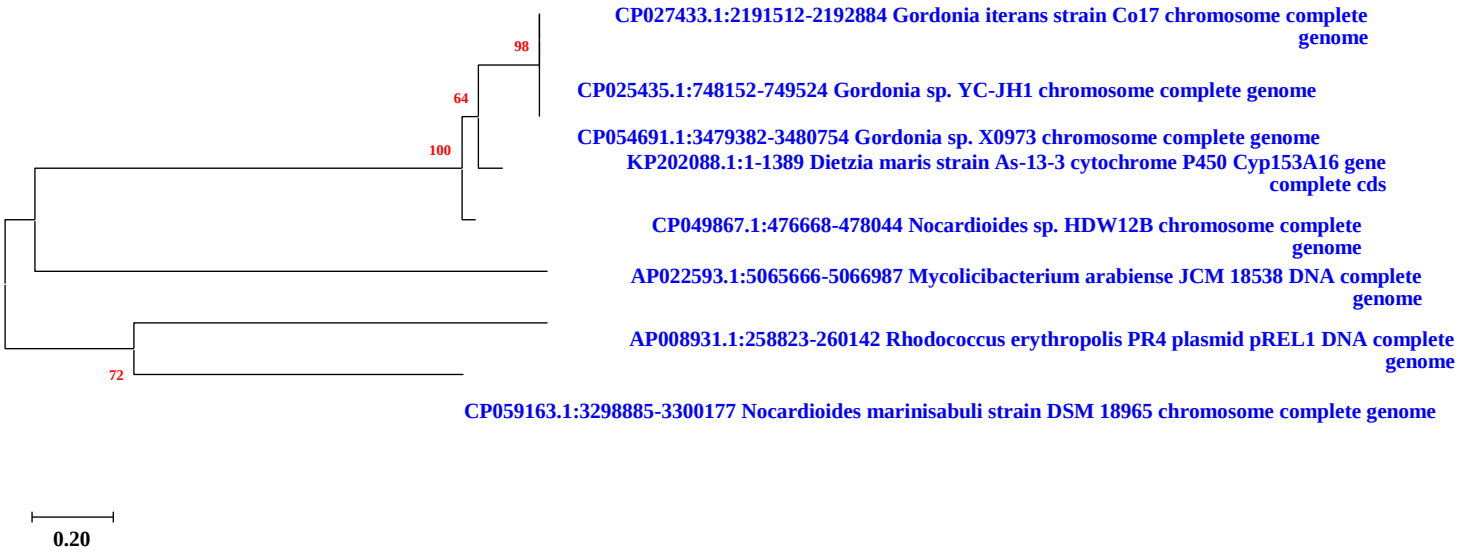


Figure 4.2: Phylogenetic relationship of *Dietzia maris* with its most related homologues using the Neighbor-joining method with 1000 bootstrap replicates

4.3 Translation of Gene into Protein and Detection of Protein's Nature

The longest open reading frame (ORF) sequence was selected starting from methionine (M), regarded as starting point, and ending at stop codon denoted by hyphen (-). It was consisting of 462 amino acids. The protein was started from nucleotide sequence ATG and ended at GGA expressed as M and G in the compact form respectively. AcalPred showed more probability towards alkaline enzyme by giving the value of 0.996374 which more than the value of acidic enzyme 0.003626.

4.4 Functional Domain Prediction and Identification of Motifs

It showed that P450 can interact selectively and non-covalently with iron ions and heme. It can catalyze the redox reaction by transferring electrons and by adding and subtracting oxygen into certain compound and water molecule respectively. InterProscan was operated to predict the motifs(Quevillon *et al*., 2005). It gave the total number of amino acids (462) present in Cytochrome P450, family relationship which includes homologous sequences and conserved

y structures are presented as Strands with yellow color, Helix with pink color and

sites. It also predicted the functions of Cytochrome P450. Cytochrome P450 takes part in redox reaction as a biological process while molecular functions that were predicted are iron ion bonding, oxidoreductase activity and the most important one is heme binding.

4.5 Secondary Structure Prediction

Secondary structure of protein plays an important role to maintain the structure and folding of proteins. Protein-Specific Iterative Basic Local Alignment Search tool (PSI-blast) based secondary structure PREDiction (PSIPRED) was used for the graphical secondary structure prediction of targeted protein Secondary structure prediction is shown in the Figure 4.3 in which helix, strands and coils are represented with different colors (McGuffin *et al*., 2000).

Figure 4.3: Secondar
Coils with gray color

4.6 Model Generation and Refinement

I-Tasser showed top 5 structures of protein with C-scores in results. The first one with C-score -0.89 was preferred and shown in the Figure 4.4. The TM-score for this model was 0.06 ±0.14 and RMSD 9.2 ±4.6A^{o}. I-Tasser showed that Cytochrome P450 had less solvent accessibility as it had more buried residues in its tertiary structure. Binding sites or active sites of protein were also predicted for residue interaction utilizing the I-Tasser results. After predicting results of Tasser, the alkaline nature of enzyme was predicted via AcalPred. It showed more probability alkaline value 0.996374 than acidic value 0.003626.

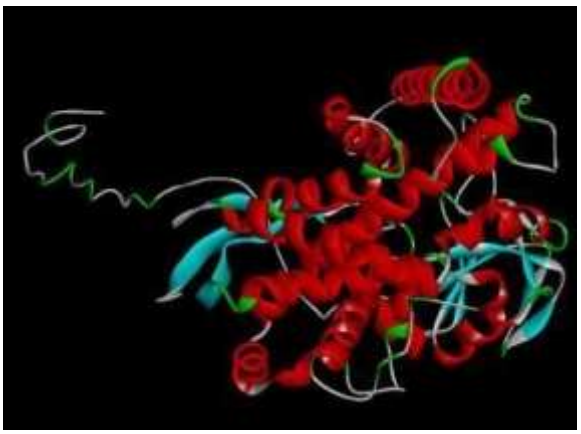


Figure 4.4: Cartoon representation of cytochrome P450 tertiary structure Refining was done by taking advantage of GalaxyRefine (Giardine *et al*., 2005) and a strong model was generated free from steric hindrance and is given in the Figure 4.5.

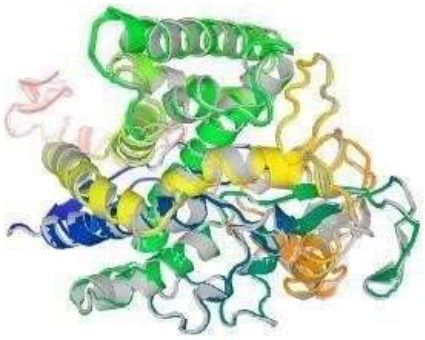
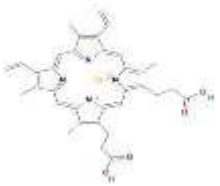
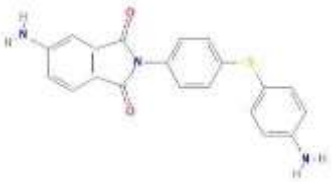
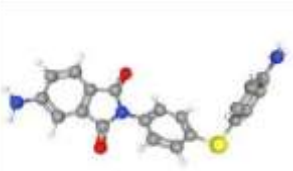


Figure 4.5: Refined model of Cytochrome P450 with RMSD 0.447 and Mol probability 2.248 Various chemicals were given in I-Tassser's results that could act as ligand for binding with target protein. The list of these ligands is given in the Table 4.2

Table 4.2*: Secondary and tertiary structures of ligands that can bind with Protein*

Ser. No	**Ligand name**	**2-D structure**	**3-D structure**
1.	Heme		

2.	(4-Hydroxy-3,5-Dimethylphenyl) (2Methyl-1-Benzofuran-3-Yl) methanone		
3.	(3R,4S,5S,7S,9E,11R,12R)-4-[(2R,3S,4R,6S)-4- (Dimethylamino)-3hydroxy6methyloxan-2-yl]oxy-12ethyl- 3,5,7,11-tetramethyl-1oxacyclododec- 9-ene-2,8-dione		
4.	5-Amino-2-{4-[(4-aminophenyl) sulfanyl] phenyl}-1H-isoindole-1,3(2H)-dione		

4.7 Protein's Properties Statistics and Hydropathy Plot

Pepstats showed molecular weight 52578.27 with 462 residues. Average weight of resiude was 113.806 with -6.5 charge. Isoelectric point was 5.3970 and improbability of expression in inclusion bodies was 0.513. It also gave reuslts about residues in which 101 tiny residues (A+C+G+S+T) with mol% 21.861, 219 small residues (A+B+C+D+G+N+P+S+T+V) with mol% 47.403, 140 aliphatic residues (A+I+L+V) with mol% 30.303, 47 aromatic residues (F+H+W+Y) with mol% 10.173, 258 non-polar residues (A+C+F+G+I+L+M+P+V+W+Y) with mol% 55.844, 204 polar residues (D+E+H+K+N+Q+R+S+T+Z) with mol% 44.156, 132 charged

residues (B+D+E+H+K+R+Z) with mol% 28.571, 65 basic residues (H+K+R) with mol% 14.069 and 67 acidic residues (B+D+E+Z) with mol% 14.502 were included. Hydropathy plot is given in the Figure 4.6.

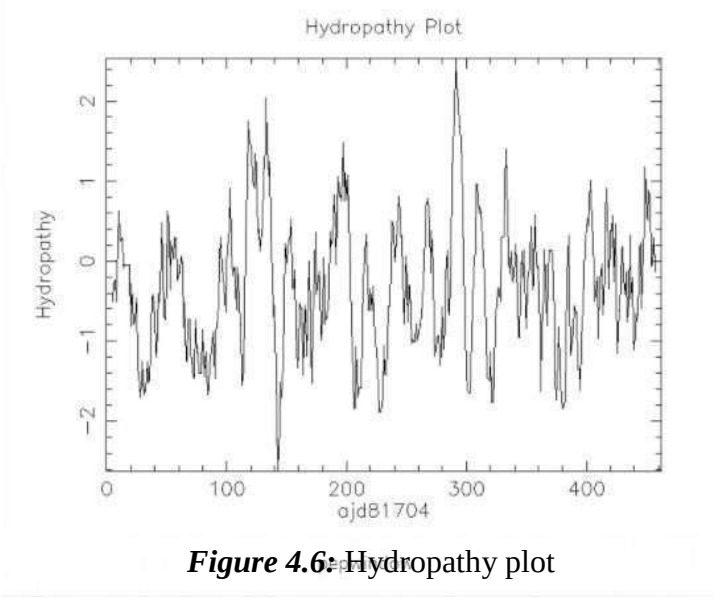


Figure 4.6: Hydropathy plot

4.8 Model Validation and Ligand Selection

Model validation was done by generating Ramachandran plot (Figure 4.7) using PROCHECK (Laskowski *et al*., 2006). Ramachandran plot obtained from PROCHECK showed that the 304 residues (76%) were present in most favored region that were A, B and L. 86 residues (21.5%) were in additional allowed region represented by a, b,1 and p. 4 residues (1.0%) were in generously allowed region revealed by ~a,~b,~l, and ~p whereas 6 residues (1.5%) were in disallowed region. Adding this up total number of non-glycine and non-proline residues could be calculated to be 400 (100%). Apart from this end-residues, 29 glycine residues (shown as triangles) and 32 proline residues were made up a total 462 residues. The secondary structure of polycarbonate and phenol formaldehyde found from PubChem and downloaded in SDF file for docking.

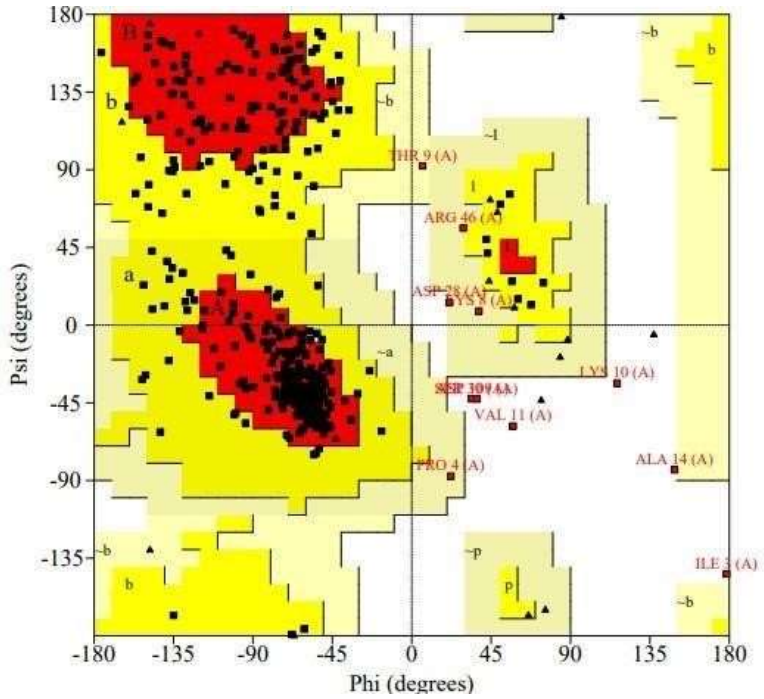


Figure 4.7: Ramachandran analysis of predicted protein with highlighted residues

4.9 Toxicity Prediction of Ligands

The molecular weight of polycarbonate was 228.29. ECOSAR showed 3.64 selected log kow value, 3.64 estimated log kow value and 3.32 measured log kow value. It predicted 120mg/L selected water solubility, 85.28mg/L estimated water solubility and 120mg/L measured water solubility. Melting point was also determined which was 153^{o}C for both selected and measured meting point. The molecular weight of phenol formaldehyde calculated was 94.11. ECOSAR predicted 1.51 as selected and estimated log kow value and 1.46 as measured log kow value. It showed 82800mg/L selected water solubility, 6084.39 estimated water solubility and 82800 measured water solubility. The melting point calculated as selected and measured meting point was 40.9

4.10 Molecular Docking

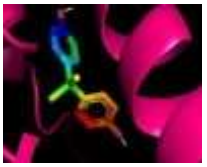
Auto dock vina was operated for interaction analysis (Trott and Olson, 2010). Cytochrome P450 showed effective interactions within the range of -5.8 to -6.7 for polycarbonate. Five residues were involved in the interaction of polycarbonate and Cytochrome P450 with highest energy of

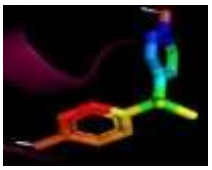
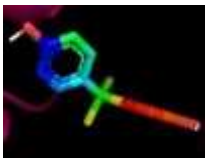
-6.7A$^{o.}$ However, it showed more higher energies than phenol formaldehyde, ranged between 4.4 to -6.0^{o}A for phenol formaldehyde. Cytochrome P450 showed highest energy of -6.0^{o}A for phenol formaldehyde degradation which was involved two residues.

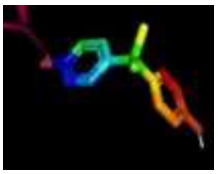
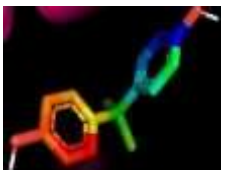
4.11 Visualization and Interaction Studies

PyMol was used for the visualization of interactions between targeted protein and ligands (Seeliger and de Groot, 2010). It showed the points of interactions on protein cytochrome P450 with ligands polycarbonate and phenol formaldehyde. The nine outcomes of docking were visualized one by one with the targeted protein cytochrome P450 for both polycarbonate and phenol formaldehyde. The visualization results of PyMol for polycarbonate and cytochrome P450 are shown in the Table 4.3. The results of PyMol for phenol formaldehyde and cytochrome P450 are shown in the Table 4.4.

Table 4.3: *Visualization of Polycarbonate and P450 Interactions with energies.*

Sr No.	**Affinity (kcal/mol)**	**Visualization**
1.	-6.7	

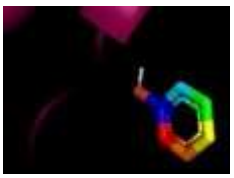
2.	-6.4	
3.	-6.3	
4.	-6.2	

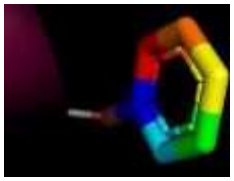
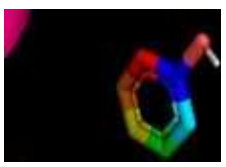
5.	-6.2	
6.	-6.1	
7.	-6.0	

8.	-5.9	
9.	-5.8	

Table 4.4: *Visualization of Phenol formaldehyde and P450 interactions with energies.*

Sr. No.	Affinity (kcal/mol)	Visualization
1.	-6.0	
2.	-6.0	
3.	-5.8	

4.	-4.8	
5.	-4.6	
6.	-4.6	

7.	-4.4	
8.	-4.4	
9.	-4.4	

Later on, discovery studio was practiced for detailed studies of interactions between protein cytochrome P450 and ligands polycarbonate and phenol formaldehyde (Wang *et al*., 2015). Protein expressed five active sites or residues for the interaction with polycarbonate and they are shown in the Table 4.5 with residues names, type of interactions, protein groups and distance between ligand and residue.

Table 4.5: *Polycarbonate interacting residues of Cytochrome P450 from Dietzia maris with distance and types of interaction*.

Sr. No.	Residue	Distance	Category	Type	From chemistry	To chemistry
1.	MET222	3.678102	Other	Pi-Sulfur	Sulfur	Pi-Orbitals
2.	MET222	3.660982	Other	Pi-Sulfur	Sulfur	Pi-Orbitals
3.	ILE123	5.181381	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl

4.	PRO126	5.237774	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl
5.	PRO127	4.329715	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl

Phenol formaldehyde, P450 expressed two interacting active sites. The residues names with the same detail are given in the Table 4.6. Interactions of ligands with cytochrome P450 are visually shown in the Figure 4.8

Table 4.6: *Phenol formaldehyde interacting residues of Cytochrome P450 from Dietzia maris with distance and types of interaction.*

Sr. No	Residue	Distance	Category	Type	From chemistry	To chemistry
1.	VAL441	2.380543	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
2.	ARG447	3.774390	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl

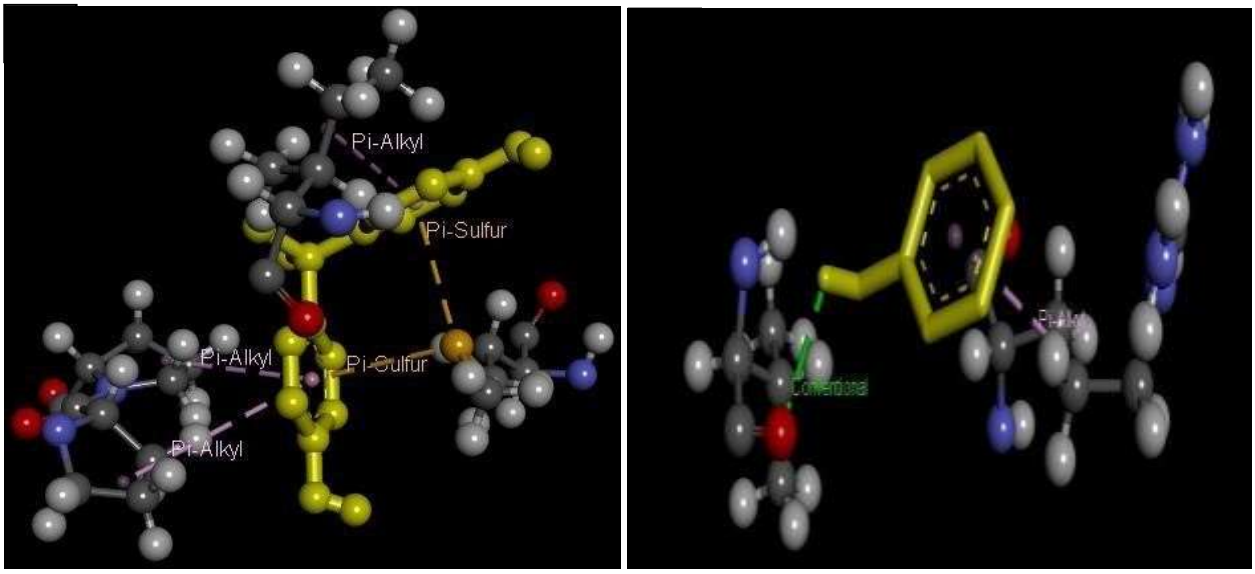


Figure 4.8: A. Interaction between polycarbonate and cytochrome P450. B. Interaction between Phenol formaldehyde and cytochrome P450

4.12 Cloning and Expression Vector

pUC-19 plasmid contains multiple cloning sites. Two different restriction sites Bpu10I and EagI were insert the gene. The coding region of gene was used for cloning purpose. The coding sequence of gene is shown in the Figure 4.9. The whole pUC-19 vector with inserted gene is shown in the Figure 4.10. The gene is inserted just after the origin of replication. The complete process in shown in the Figure 4.11.

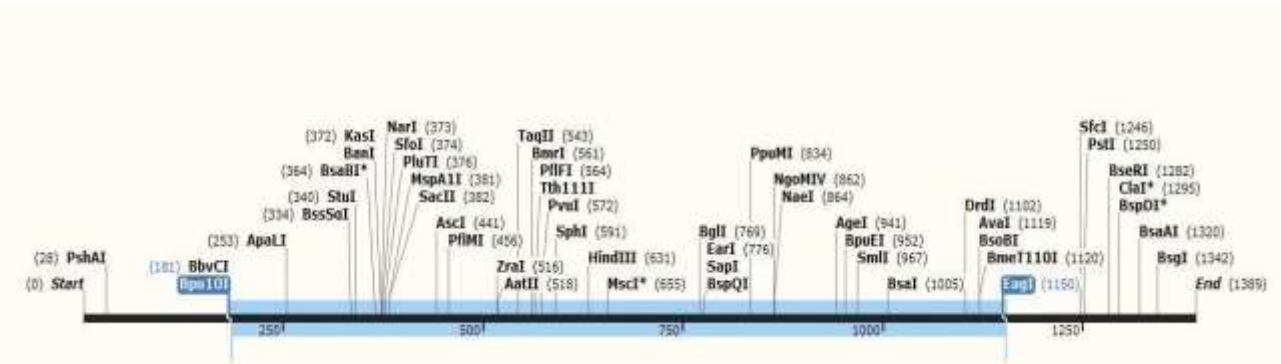


Figure 4.9: KP202088.1 *Dietzia maris* strain As 13-3 cytochrome P450 Cyp153A16 gene

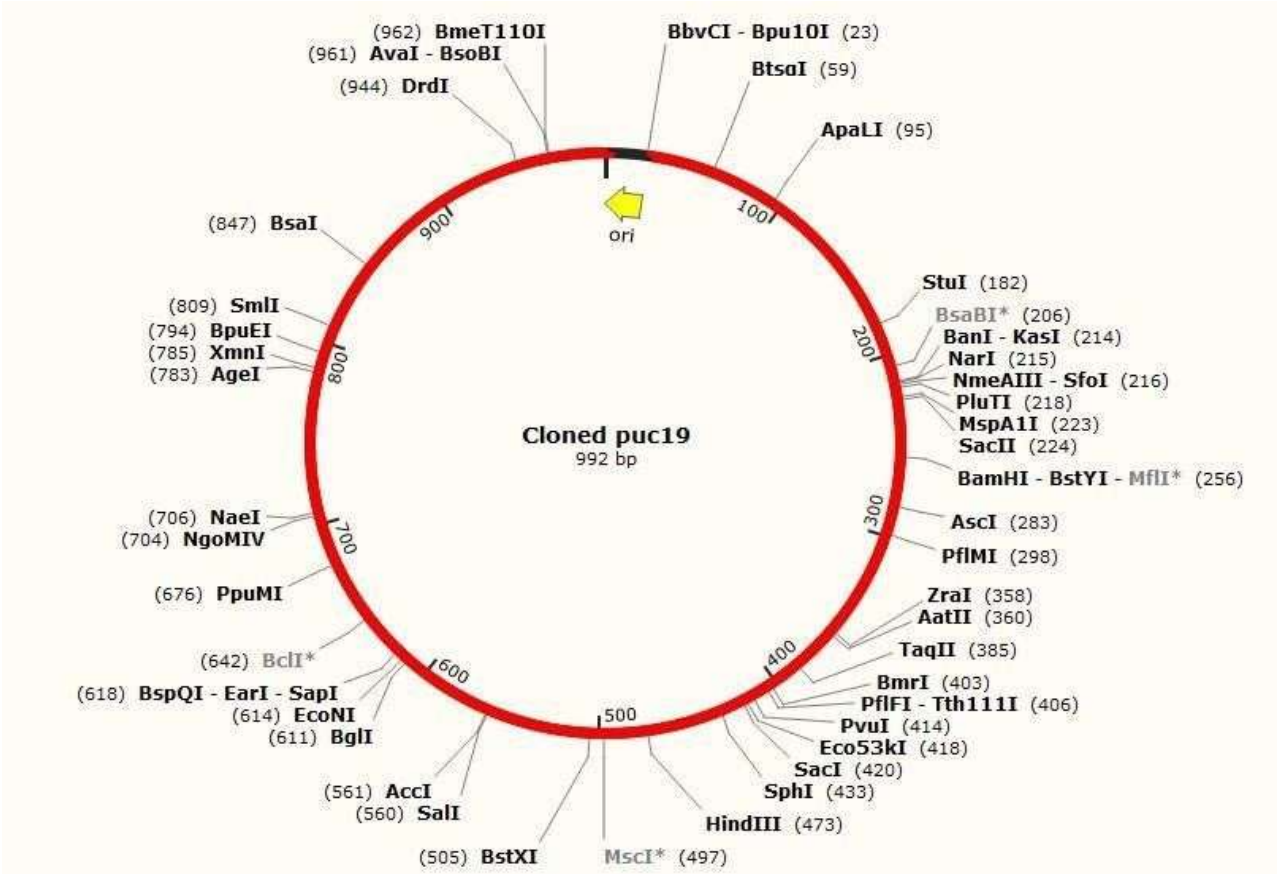


Figure 4.10: pUC-19 plasmid with Cytochrome P450

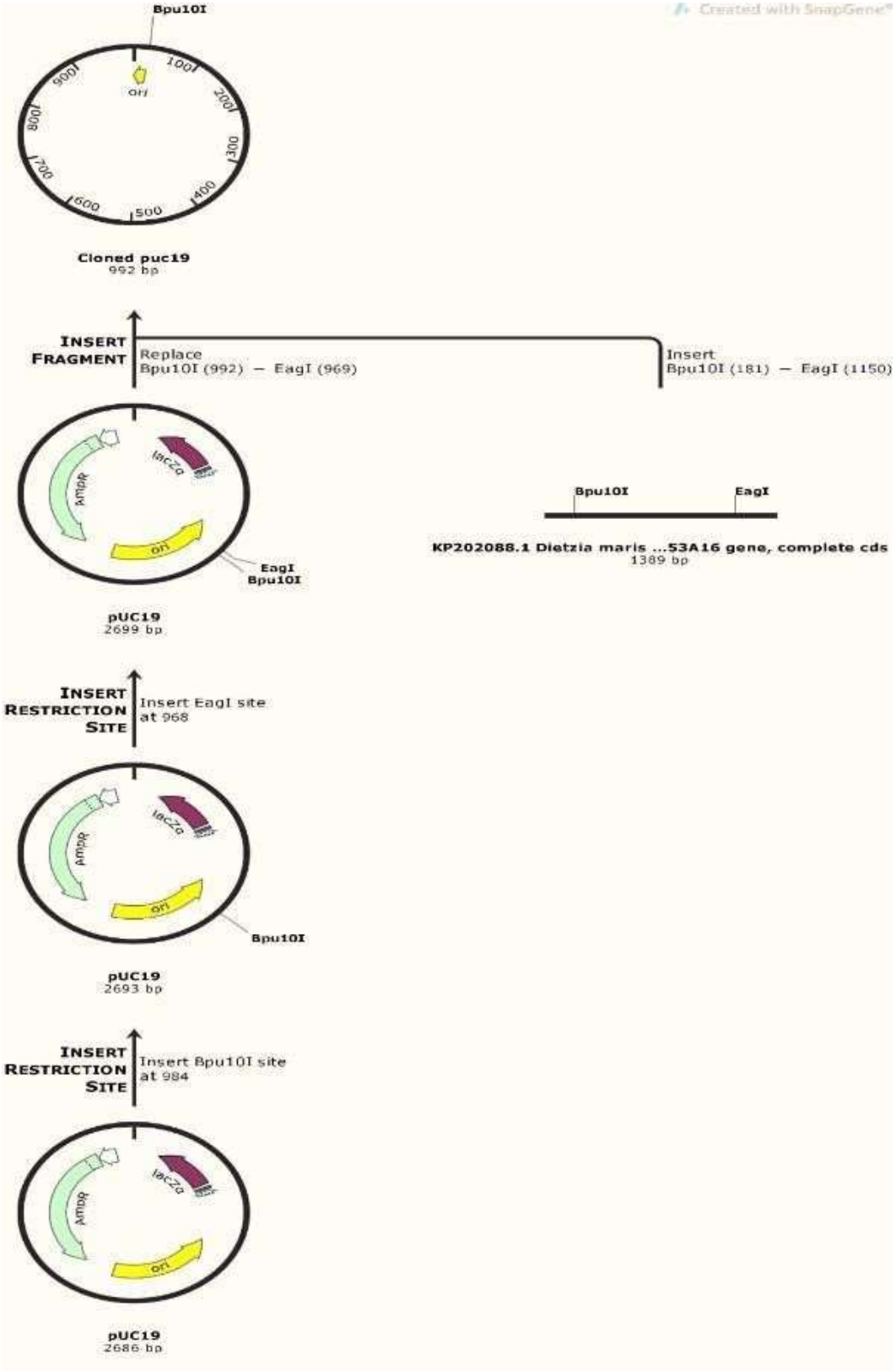


Figure 4.11: Snap-Gene session history

CHAPTER FIVE: DISCUSSION

In this time of plastic pollution, it is the need of hour to find out a suitable treatment to control the plastic pollution. In this modern and advanced time of computer aided, bioinformatics and many computational tools are helpful to check the degrading ability of certain enzyme. *In-silico* degradation of various other pollutants have already been seen effective and given good results via computational tools (Díaz, 2004) . Plastic is made up of polymers and are resistant against microbial attacks. Polymer chain makes back bone of the molecule which not easily degradable (Singh and Sharma, 2016).

However, the physiochemical traits exposed the high suitability of protein to degrade the plastic (Jincy *et al*., 2017). Protein structure was not available on PDB, so designed though bioinformatics tool and also validated to check its authenticity. In short, this study was directed to the degradation of plastic made up of polycarbonate and phenol formaldehyde polymers to treat the plastic pollution by operating computational tools. Cytochrome P450 protein coding enzyme was used for the degradation of plastic.

In the past, some harmful technologies were applied like landfilling, incineration and marine disposal for the disposal of plastic (Alabi *et al*., 2019). Also, the same enzyme cytochrome P450 was used for the degradation of oils (Wang *et al*., 2014b). But the protein has not been used before for the degradation of polycarbonate and phenol formaldehyde. With the developments in the field of bioinformatics, treating the pollutants with microorganisms or their enzymes at *in-silico* level is the modern field of environmental biotechnology. It helps researchers to check the effectiveness of any enzyme of protein against some receptors before going towards *in-vitro* level.

In order to check the effectiveness of cytochrome P450, all the crucial steps were performed

before docking. Protein's alignment was determined to predict the similar sequences using BLASTn and ClustalW. Phylogenetic analysis was done to check the ancestral history and the origin of gene. The gene was translated into protein to check its interaction with the ligands. AcalPred determined that there is a higher probability of enzyme being alkaline (Deyev *et al*., 2015). InterProscan was utilized to predict the functional domains of protein. Secondary and tertiary structures were generated via PSIPRED and I-Tasser respectively. Protein's purification was done through Galaxy refine for the proper functioning of protein. Four other ligands that showed effective binding with protein were also focused.

Furthermore, analysis of protein properties at statistics level were also mentioned which are helpful to determine the distances and the angles between various residues it also gave information about the total number of different size residues. Hydropathy plot was designed through Pepwindows to reveal the hydrophobicity of protein over the length of nucleotide sequences (White, 1994). For protein's validation, Ramachandran plot was drawn via PROCGECK to reveal the unrealistic facts present in the protein's model such as psi and phi bonds.

Toxicity of ligands were determined to predict their bad effects on the environment and living organisms. ECOSAR was operated to obtain the valuable results as it is a most common *insilico* tool to analyze the impacts any harmful pollutants on environment and various animals. Different organisms in the oceans were the major victim of plastic pollution. Polycarbonate showed logKow value 3.64 and phenol formaldehyde 1.51, although both are less harmful but it does not mean that they are not harmful. Polycarbonate can disturb reproductive system by changing the normal estrogen level and it can also cause cancer (Fukuoka *et al*., 2003). On the other hand, phenol formaldehyde can cause skin burn, harmful allergies and inhalation problem (Boreiko *et al*., 1982). Both can cause malnutrition and suffocation in marine animals. Focusing on docking as it is a good method to detect the effective substrate of enzyme, it can

be utilized to enhance the check the activity of enzyme for various pollutant degradation (Liu *et al*., 2018). Cytochrome P450 can also be highlighted in the list of pollutant degrading enzymes. So, the final step docking was performed and it showed effective results for the plastic degradation by using cytochrome P450. The highest binding energy was seen in the case of polycarbonate was –6.7^{o}A and that of phenol formaldehyde was -6.0^{o}A.

PyMol was used for the visualization of position of interacting ligands with protein (Seeliger and de Groot, 2010). The number of residues of polycarbonate and phenol formaldehyde interacting with Cytochrome P450 were identified through Discovery studio and it showed five and 2 residues for poly carbonate and phenol for n aldehyde respectively. The type of interactions is and the distance between residues and protein were also mentioned in the Table 4.5 and 4.6.

In this study, gene cloning of cytochrome P450 gene into an expression vector have been also discussed. The microbe can be directly combined with the polluted sites and also the enzyme isolated from microbe can be utilized to decompose the plastic waste. Microbes such as bacteria and fungi grow rapidly and are more susceptible to genetic manipulation (Harvey, 2009). Due to this reason, cytochrome P450 was used to clone into plasmid vector named as pUC-19. It can be expressed in same bacteria that is *Dietzia maris* to enhance the expression of and at large scale and the process can be accelerated. It can easier the isolation of gene from microorganism.

This method has various advantages if it is applied at large scale degradation. It is high time to use ultra-modern technologies like Blockchain in Health Care Supply Chain Management like Arab (Sakib, 2022) or Robotics in Health Care (Sakib, 2022).

CHAPTER SIX: CONCLUSION AND FUTURE PERSPECTIVE

In conclusion, cytochrome P450 has shown encouraging results for the degradation of polycarbonate and phenol formaldehyde. Degradation affinity is dependent upon various

factors like pH, temperature, reaction time and the dose of enzyme. Significant results are obtained for affinity with both types of plastic. It was predicting that the both pollutants are potential substrates for cytochrome P450 and also need to be treated because they are very harmful for living organisms and the environment. This *in-silico* method can be adopted for the detoxification of many other pollutants found in the environment. Further studies are desired for the detoxification of pollutants and expression of gene in microorganism at *in-vitro* level.

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